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Volume 11 July 15, 1961 No. 3

COMMENTS ON THE NOMENCLATURE OF THE CAUSATIVE AGENT OF TULAREMIA

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SUMMARY: The organism causing tularemia was named Bacterium tularense by McCoy and Chapin in 1912. It has been placed in several bacterial genera in recent years. It is termed Pasteurella tularensis (McCoy and Chapin) Bergey et al. in the 7th (1957) edition of Bergey's Manual of Determinative Bacteriology. This study confirms the designation of this organism as the type species of the genus Francisella Dorofeev 1947 with the species name Francisella tularensis (McCoy and Chapin) Dorofeev 1947.

For some years, the bacterial organism causing tularemia, originally named Bacterium tularense in 1912 by McCoy and Chapin, has had an uneasy and often criticized systematic assignment to the genus Pasteurella Trevisan 1887, in which it has been included in the last edition of Bergey's Manual of Determinative Bacteriology (Breed and collaborators, 1957) as Pasteurella tularensis (McCoy and Chapin, 1912) Bergey et al., 1923.

Olsuf'ev et al. (1959) have recently stated their opinion that Dorofeev (1947) was correct in erecting the genus Francisella to contain this organism. The former also proposed two geographic varieties based on biological strain differences which are not pertinent to this discussion.

In a later description of the etiologic agent, Olsuf'ev and Rudnev (1960) correctly point out that Dorofeev's subsequent

proposal (1956) to substitute Tularecella as the preferred generic assignment was invalid under the international rules. Since Francisella Dorofeev, 1947, was validly published, the author cannot arbitrarily reject the name (for example, by invoking Rule 12c) to propose another presumably more suitable name (Tularecella).

Three questions arise in deciding nomenclatural assignment of this species, since it is accepted as not belonging in Bacterium sens. str.: 1) What is the type species of the genus Pasteurella; 2) is tularensis congeneric with it; and if not, 3) is Francisella the first availabe genus to which the species can be properly assigned?

No doubt the original description of a "plague-like organism causing disease in rodents, "in an area affected by true plague caused by P. pestis (Lehmann and Neumann, 1896) Holland 1920, has contributed to attempts at maintenance of tularensis in the same generic assemblage, as has also the characteristic of bipolar staining, common to several species as now grouped.

In answer to question 1, Trevisan first erected "somewhat tentatively" (1885) the genus Octopsis with O. cholerae-gallinarum (the cause of fowl cholera, possibly in reference to Micrococcus cholerae-gallinarum Zopf 1885) as the first of five included species. Two years later Trevisan (1887) abandoned this genus in favor of the new name, Pasteurella, which was validly published to contain the only associated species P. cholerae-gallinarum. Because of this, the International Judicial Commission in Opinion 13 (1954, Internat. Bull. Bact. Nomen. Tax., 4: 153) placed Octopsis in the list of rejected generic names while Pasteurella was placed in the list of conserved generic names. Buchanan and Buchanan (1938) had included this organism and P. tularensis in their discussion of Pasteurella.

Since Pasteurella is monobasic for P. cholerae-gallinarum (Zopf) Trevisan, the assignment of P. "multocida (Lehman and Neumann, 1899) Rosenbusch and Merchant 1939"*

*Actually Rosenbusch and Merchant (1939, p. 69) ascribe the typical agent to the binary combination "Pasteurella multocida, Kitt, 1885, n. comb."! But Kitt's original report (1885) provides neither a valid, new binary name for this bacterial agent nor the inadmissible ternary one, Bacterium bipolare multocidum, credited to him. P. multicoda

in the Bergey system as type is valid only in the sense that the latter is accepted as an objective synonym of the former, which it appears to be. Nor can the type be either "P. avicida [Prévot]" or "Pasteurella aviseptica [Top. and Wilson]" cited by Hauduroy et al. (1953). Hughes (1930) includes P. cholerae gallinarum [sic] as one of 5 synonyms of the later P. avicida, while Quortrup et al. (1946) discussed variants of the species.

On the basis of special growth requirements on enriched media, the tularemia agent does not fit the restrictive characters of the other species, including the type, now assigned to Pasteurella. This answers in the negative question 2 about congeneracy.

It is stated in standard texts that P. tularensis does not grow anaerobically, which has been confirmed in recent experiments by us (unpublished data) with old laboratory-adapted and recently isolated strains. This is not a critical criterion for maintaining P. tularensis in the genus Pasteurella, because many authorities ignore this characteristic and still consider that P. tularensis is not properly grouped in the assemblage of species under Pasteurella, for example, Hagan and Bruner (1951, p. 236).

Two other genera have been synonymized under Pasteurella, namely Coccobacillus Gamaleĭ 1888 and Eucystia Enderlein 1917. Gamaleĭ described "the Coccobacillus with specific designation avicidus" (transl.) for the "avian cholera bacteria" which caused "avian septicaemia," based on isolates from pigeons. These isolates were related immunologically to strains obtained from the Pasteur Institute. Coccobacillus appears to have been validly published at that time, but the synonymy of the agent under Pasteurella would seem to be correct and hence would not apply to P. tularensis.

Eucystia was apparently validly published, even though under a family Bacteriidae (with improper zoological ending) in a key with only four dichotomically arranged characters, and despite the fact that the designated type species, E. pestis, was wrongly ascribed to Yersin 1894 who described but did not name the plague organism. Whether this species

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therefore, was proposed and characterized (p. 85) by the former authors, though they attributed it gratuitiously to Kitt in the genus Bacterium. This name cannot supplant the above, original legitimate type species for the genus Pasteurella.

is assignable to Pasteurella, as considered by Buchanan and Buchanan (1938), is not within the province of this discussion, but the name Eucystia will come into considerations of priority in connection with Pesticella Dorofeev 1947, described for the same agent. Even if the concept of Pasteurella is refined to include P. pestis in a subgenus, Eucystia, such restriction would still relegate P. tularensis to a different subgenus on biological grounds.

Some authors have assigned P. tularensis to the genus Brucella but this has not been widely accepted, and again the agent is excluded on its biological properties. For similar reasons, the agent cannot be included under Coccobacterium Klinger 1912 (preoccupied by Schmidt and Weis) as Olsuf'ev et al. (op. cit.) have pointed out when they also cite another name, Cystinophilus, which was verbally and not validly proposed by Elbert in 1939.

In view of the need for revised generic assignment of P. tularensis, it appears that Olsuf'ev and his colleagues are correct in asserting that Francisella Dorofeev 1947 is the first available restrictive generic name. Valid publication of this genus was verified in a copy of the reference supplied to R. E. Buchanan by Professur Olsuf'ev in which the binary combination in Latin, "Francisella tularense McCoy and Chapin 1910" was designated type species. The proper type species reference is, therefore, Francisella tularensis (McCoy and Chapin) Dorofeev. The name, Francisella, is equally available should subgeneric treatment ultimately be proposed on taxonomic grounds.

Since cultures of the original isolater are no longer available it will be difficult, if desirable, to select one of the present-day isolates as a neotype culture in view of the now well-known variation in biological properties.

Olsuf'ev and Rudnev proposed that Francisella should properly be placed in the latest Bergey system between the genera Brucella and Pasteurella in the family Brucellaceae of the order Eubacteriales. We agree with this and suggest the following revision in the dichotomous key to the Brucellaceae:

c. Attacks carbohydrates
  d. Grows on ordinary media. . Genus Pasteurella
  dd. Grow only on special media. . Genus Francisella
cc. Carbohydrates not attacked. . . Genus Brucella

BIBLIOGRAPHY

Bollinger, O. 1885. Zur Aetiologie des Milzbrandes. Sitz. Gesell. Morph. u. Physiol., München. 1: 21-26.

Breed, R. L. and collaborators. 1957. Genus Pasteurella in Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore. 7th ed. pp. 395-402.

Buchanan, R. E. and E. D. Buchanan. 1938. Bacteriology. MacMillan Co. 4th ed. 548 pp.

Dorofeev, K. A. 1947. On classification of tularemic bacteriae. Symposium of Research Works of Inst. Epidem. Mikrobiol. in Chita. 1: 177-178.

_____. 1956. Tularemia (tularetsellez) in animals. Avtoref. diss. Leningrad (cited by Olsuf'ev, 1960).

Enderlein, G. 1917. Ein neues Bakteriensystem auf vergleichend morphologischer Grundlage. (Bakteriologische Studien IV). Gesellsch. Naturforsch. Freunde zu Berlin, Sitzungsberichte. pp. 309-319.

Gamaleiă, N. 1888. Zur aetiologie der Hühnercholera nebst einigen Bermerkungen über die Schutzimpfungsfrage. Centrl. f. Bakt., 4: 161-168.

Hagan, W. A. and D. W. Bruner. 1951. The Infectious Diseases of Domestic Animals. 2nd Edition, Comstock Publishing Company, Inc., Ithaca, New York. 920 pp.

Hauduroy, P., Ehringer, Guillot, G., Magrou, Prévot, A. R., Rosset and Urbain. 1953. Dictionnaire des bactéries pathogènes pour l'homme, les animaux et les plantes. 2nd ed. Masson, Paris. 692 pp.

Hughes, T. P. 1930. The epidemiology of fowl cholera. II. Biological properties of P. avicida. J. Exp. Med., 51: 225-238.

Kitt, T. 1885. Ueber eine experimentelle, der Rinderseuche (Bollinger) ähnliche Infectionskrankheit. Sitz. Gesell. Morph. u. Physiol., München. 1: 140-168.

Klinger, P. 1912. Ueber eine neuen pathogenen Anaeroben aus menschlichem Eiter (Coccobacterium mucosum anaerobicum n. sp.). Zentralbl. Bakt. I. Abt. Orig., 62: 186-191.

Lehman, K. B. and R. Neumann. 1899. Atlas und Grundriss der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik. München. 2 Aufl., 2: 495 pp.

McCoy, G.W. and C.W. Chapin. 1912. Bacterium tularense the cause of a plague-like disease in rodents. Pub. Health Bull. No. 53: 17-23.

Olsuf'ev. N.G., O.S. Emelyanova and T.N. Dunayeva. 1959. Comparative study of strains of B. tularensa in the Old and New World and their taxonomy. J. Hyg., Epidem., Microbiol., and Immunol., 3: 138-149.

_____ and Rudnev, G.P. (editors). 1960. Tularemia. Medgiz, Moscow. 459 pp.

Quortrup, E.R., F.B., Queen and L.J. Merovka. 1946. An outbreak of pasteurellosis in wild ducks. J. Am. Vet. Med. Assoc., 108: 94-100.

Rosenbusch, C.T. and I.A. Merchant. 1939. A study of the hemorrhagic septicemia Pasteurellae. J. Bact., 37: 69-89.

Trevisan de Saint Leon, V. 1885. Caratteri di alcuni nuovi generi di Batteriacée. Atti della Academia Fisio-Medico-Statiscain Milano, Ser. 4, 3: 92-107.

_____. 1887. Sul Micrococco della rabbia e sulla possibilita di riconescere durante il periodo d'incubazione, etc. Rendiconti Reale Instituto Lombardo di Scienze e Lettere, Series II, 20: 87-105.

Zopf, W. 1885. Die Spaltpilze. Nach dem neuesten Standpunkte bearbeitet. Breslaw 3 Aufl., 127 pp.

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A TAXONOMIC STUDY OF THE MEMBERS OF THE GENUS SERRATIA

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SUMMARY: A study of the morphological, cultural, and biochemical characteristics of 12 strains of different species of the genus Serratia indicates that most strains of species thus far recognized are identical with Serratia marcescens.

The names S. piscatorum (Lehmann and Neumann) Breed, S. pyoseptica (Fortineau) Bergey, S. fuschsina (Boeckhout et de Vries) Bergey, S. anolium (Duran-Reynals et Clausen) are to be regarded as synonyms of S. marcescens Bizio and Serratia marinorubra as a synonym of Serratia marcescens var. kiliensis.

The strains S. saponaria I and II Markov should be removed from the genus Serratia and placed provisionally in the genus Alcaligenes.

For our studies of the species of the genus Serratia, we have obtained from various collections cultures of strains without designation of species or with obsolete names. Among these cultures were strains names, e.g. Serratia pyoseptica (Fortineau) Bergey, Serratia anolium Duran-Reynals and Clausen, Serratia fuchsina (Boeckhout and de Vries) Bergey and others. Breed (1948) regards most of these names as synonyms of Serratia marcescens, while Krasilnikov (1949) includes them in his Manual as names of accepted species.

In the 1957 edition of Bergey's Manual neither of the two new strains of Serratia, isolated and described by Markov (1956) as Serratia saponaria I and II, is included.

In our present work we have studied whether the several aforesaid "species" have taxonomic significance and whether some of them belong to the genus Serratia at all.

MATERIAL AND METHODS

In our work we have thoroughly studied 12 strains of Serratia acquired from various collections. The list of strains is given in Table 1.

The methods used were the same as in our previous work (Martinec and Kocur, 1960).

Table 1.

No. of strain	Source of strain
Serratia anolium	ATCC 6065
S. anolium B 1700	W.C. Haynes, NRRL, Peoria, Illinois
S. pyoseptica S 4	Dept. of Appl. Biol. NRC, Ottawa, Canada
S. pyoseptica 523	J. Simpson, Prairie Reg. Lab., Saskatoon, Canada
S. saponaria I. II.	W. Markov, Sofia, Bulgaria
S. fuchsina 150	B. Hampl, Dept. of Biol. Sci. Prague
S. marinorubra 318	C.E. Zobell, La Jolla, Calif.
S. piscatorum 415	Inst. of Plant Product., Prague
Serratia sp. S 13, S 26	J. Simpson, Prairie Reg. Lab. Saskatoon, Canada
Serratia sp. 846	C.B. van Niel, Stanford Univ., California
Alcaligenes faecalis 09	Inst. of Epidemiology and Microbiol., Prague

RESULTS

Morphology. All studied strains were short, gram-negative rods occurring singly and in clumps. Size of individual cells 0.8-1 x 1.5-2.5 μ. All strains were motile except Serratia saponaria II.

Cultural characteristics. Colonies of most strains were round, with undulate margin, slightly convex, smooth. Eight strains were at first creamy or slightly rose colored, later red, very slightly pigmented was S. saponaria I. and there were three non-pigmented strains S. saponaria II., no. 6065 and B 1700. In nutrient broth all strains produced turbidity and sediment except S. saponaria I. and II. which grew very poorly. S. saponaria I. and II., however, grew well in nutrient broth at pH 9.6. The mentioned strains did not grow on potato. On the medium recommended by Dewey and Poe (1943) 6 strains only were pigmented.

Biochemical characteristics. The ascertained biochemical characteristics are shown in Table 2.

DISCUSSION

The results indicated that all the strains with the exception of Serratia saponaria I and II agreed with the characteristics of Serratia marcescens Bizio. The differences as shown in Table 2 (lactose fermented by strain 318, gas not formed from glucose by strains 523, 318, S 26, maltose not fermented by strain S 4 and adonitol not fermented by strain 527) are characteristic for Serratia marcescens. Serratia marinorubra BS 318 (acethylmethylcarbinol not formed) is identical with Serratia marcescens var. kiliensis.

We conclude that the aforesaid species S. anolium Duran-Reynals and Clausen, S. pyoseptica (Fortineau) Bergey, Serratia fuchsina (Boeckhout and de Vries) Bergey are to be regarded as synonyms of Serratia marcescens Bizio, and Serratia marinorubra as a synonym of Serratia marcescens var. kiliensis.

Serratia marcescens differs significantly from Serratia saponaria I and II. The differences in the biochemical activity of these two strains in comparison with the strain Serratia marcescens are so substantial, that we conclude that these two strains should not be placed in the genus Serratia. We have compared biochemical characteristics of Serratia saponaria I and II with one strain of Alcaligenes faecalis 09 and with the characteristics of this species in Bergey's Manual (1957). We have ascertained that biochemical features of Serratia saponaria I and II and Alcaligenes faecalis are very similar (liquefaction of gelatin, no fermentation of carbohydrates, growth at pH 9.6). On the

Diagnostic Tests Used	S. anolium 6065	S. anolium B 1700	S. pyoseptica S 4	S. pyoseptica 523	S. saponaria I.	S. saponaria II.	S. fucheina 150	S. marinorubra 318	S. piscatorum 415	Serratia sp. S 13	Serratia sp. S 26	Serratia sp. 846
Gelatin liquefaction	+	+	+	+	+	-	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	-	-	+	-	+	+	+	+
Nitrate reduction	+	+	+	+	-	+	+	+	+	+	+	+
Hydrogen sulfide I.	+	+	+	+	-	-	+	+	+	+	+	+
Hydrogen sulfide II.	+w	+w	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-
Milk	+	+	+	+	-	-	+	+	+	+	+	+
Hemolysis	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	-	-	+	-	+	-	-	+
Gas from glucose	+	+	+	-	-	-	+	-	+	-	-	+
Lactose	-	-	-	-	-	-	-	+	-	-	-	-
Sucrose	+	+	+	+	-	-	+	+	+	+	+	+
Maltose	+	+	-	+	-	-	+	+	+	+	+	+
Galactose	+	+	+	+	-	-	+	+	+	+	+	+
Fructose	+	+	+	+	-	-	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	-	-	+	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	+	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	+	+	+	-	-	-	+	+	+	+	+	+
Adonitol	+	+	+	-	-	-	-	+	+	-	-	+
Sorbitol	+	+	+	+	-	-	+	-	+	+	+	+
Mannitol	+	+	+	+	-	-	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-
Esculin	+	+	+	+	-	-	+	+	+	+	+	+
Koser's citrate	+	+	+	+	-	-	+	+	+	+	+	+
Simmons' citrate	+	+	+	+	-	-	+	+	+	+	+	+
Acethylmethylcarbinol	+	+	+	+	-	-	+	-	+	+	+	+
Methyl red	-	-	-	-	-	-	-	-	-	-	-	-
KCN	+	+	+	+	+	-	+	+	+	+	+	+
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	+	-	-	-	-	-	-
Lipase	+	+	+	+	-	-	+	+	+	+	+	+
5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+
7.5% NaCl	+	+	-	+	+	+	+	+	+	+	+	+
Pigment	-	-	+	+	+	-	+	+	+	-	-	-

w = weak
Hydrogen sulfide I. = lead acetate papers
Hydrogen sulfide II. = modified Kliger medium

basis of this observation we recommend placing Serratia saponaria I and II provisionally in the genus Alcaligenes. The definitive placing of this species will be possible only after having compared it with other species of the genus Alcaligenes.

Special attention should be given to the species S. piscatorum (Lehmann and Neumann) Breed. This species has been recognized both by Krasilnikov (1949) and Breed (1957). But this species is not deposited in any collection and therefore it cannot be compared with Serratia marcescens. The only strain assigned to S. piscatorum, which we could compare, was isolated several years ago from soil (see Table 1). We have ascertained that this strain is identical with Serratia marcescens. On comparison of the original characteristics (1884) as well as the description in Bergey's Manual (1957) with the features of Serratia marcescens, we find only small differences. The fact that S. piscatorum can produce pigment at 37° and that this pigment is soluble in water, cannot be taken for such an essential feature, on the basis of which it would be possible to acknowledge S. piscatorum a valid species, because this feature can be found also in some strains of Serratia marcescens. We have ascertained that out of 68 strains studied at 37°C only 15 strains produced pigment (unpublished data). Also the fact pigment of some heavily pigmenting strains of Serratia marcescens is soluble in water, was stated already in 1948 by Breed and recently it has been experimentally proven by Williams et al. (1958). For these reasons we regard Serratia piscatorum (Lehmann and Neumann) Breed as a synonym of S. marcescens.

REFERENCES

Breed, R.S. and M.E. Breed. 1927. The genus Serratia Bizio. Zentralbl. f. Bakt. II. Abt. 71: 435-440.

_____, E.G.D. Murray and A.P. Hitchens. 1948. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

_____, _____ and R.N. Smith. 1957. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

Boeckhout, F.W.J. and J.J. Ott de Vries. 1898. Über einem neuen chromogenen Bacillus. Zentralbl. f. Bakt. 4: 487-501.

Davis, B.R., W.H. Ewing and R.W. Reavis. 1957. The biochemical reaction given by members of the Serratia group. Internatl. Bull. Bact. Nomen. and Taxon. 7: 151-160.

Du Bois, Saint Sévrin. 1894. Panaris des pécheurs et microbe rouge dela sardine. Ann. Inst. Pasteur. 8: 152-160.

Duran-Reynals and H.J. Clausen. 1934. A contagious tumor-like condition in the lizard (Anolis equestris) as induced by new bacterial species, Serratia anolium (sp. n.). Jour. Bact. 33: 369-376.

Fortineau, L. 1905. L'Erythrobacillus pyosepticus et les bactéries rouges. Bull. Inst. Pasteur. 3: 13-14.

Lehmann, K.B. and R.O. Neumann. 1886. Bacteriologische Diagnostik. München.

Markov, W. 1956. Serratia saponaria I. und II. Schädliche Bakterien in der Seife. Zentralbl. f. Bakt. II. Abt. 110: 26-32.

Martinec, T. and M. Kocur. 1960. The taxonomic status of Serratia plymuthica (Lehmann and Neumann) Bergey et al. and of Serratia indica (Eisenberg) Bergey et al. Internatl. Bull. Bact. Nomen. and Taxon. 10(4): 247-254.

_____, _____. 1961. The taxonomic status of Serratia marcescens Bizio. Internatl. Bull. Bact. Nomen. and Taxon. 11(1): 7-12.

Williams, R.P., W.W. Taylor, D. Hawkins and I.L. Roth. 1958. A water soluble diffusible pigment produced by a strain of Serratia marcescens (Chromobacterium prodigiosum) Nature. 182: 1028-1029.

ZoBell, C.E. and H.C. Upham. 1944. A list of Marine Bacteria including descriptions of sixty new species. Bull. Scripps. Inst. Oceanography, La Jolla. 5: 255.

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SEROLOGICAL RELATIONSHIP OF MIMEAE, MORAXELLA, DIPLOCOCCUS MUCOSUS AND NEISSERIA WINOGRADSKYI

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SUMMARY: For the proper classification of the Mimeae, Moraxella, Diplococcus mucosus and Neisseria winogradskyi, the serological relationship should be considered. In a serological comparison using strains of Moraxella, N. winogradskyi and D. mucosus against polyvalent Mima polymorpha antiserum, adsorbed polyvalent Herellea antiserum and Bacterium anitratum serotypes 1-10, it was found that one of the five D. mucosus strains was serologically the same as B. anitratum, type 7. The other four strains of D. mucosus and N. winogradskyi were agglutinated by adsorbed polyvalent Herellea antiserum and share partial antigens with certain serotypes of B. anitratum. Henriksen (1952) suggested further study was necessary before Mima polymorpha var. oxidans could be included in the genus Moraxella. In the present study, 57% of the Moraxella strains studied were agglutinated by polyvalent M. polymorpha antiserum and M. polymorpha var. oxidans (pool of 3 sera). The strain of Bacterium lwoffi var. brevis included was agglutinated only by Moraxella lacunata antiserum.

In a previous publication a method for the differentiation of the tribe Mimeae De Bord (1942) from the Neisseriae was reported (Cary et al., 1958). At that time, no strains of Moraxella, Diplococcus mucosus von Lingelsheim (Seelinger 1953) or Neisseria winogradskyi Lemoigne et al. (1952) were included. Consequently, this laboratory has

obtained many of these strains for a study of the serological relationship of these cultures to the Mimeae using polyvalent Mima polymorpha antiserum, polyvalent adsorbed Herellea antiserum, and the 10 serotypes of Bacterium anitratum described by Ferguson and Roberts (1950). It was felt that such a study was necessary for the proper classification of these organisms.

MATERIALS AND METHODS

The strains used in this study included the 10 distinct serotypes of Bacterium anitratum designated by Ferguson and Roberts (1950). The Moraxella strains were furnished by Dr. S.D. Henriksen of the State Institute for Public Health, Oslo, Norway and by Dr. W.A. Clark of the American Type Culture Collection, Washington, D.C. One strain of Neisseria winogradskyi was obtained from Dr. M. Lemoigne of the Pasteur Institute. Five cultures of Diplococcus mucosus, isolated by Dr. H. Seeliger, and one of Bacterium lwoffi var. brevis were secured from Miss E.O. King of the Communicable Disease Center, Atlanta, Ga.

Antigens for the precipitin tests using Maxted's filtrate were prepared according to the method previously described (Cary et al., 1956) except for the following modification to insure a sufficient quantity to perform all the tests desired. The cultures of Moraxella were grown on freshly prepared Casman's blood agar at 37°C for 18 hr under 10% CO_2 to prevent drying. All the other cultures were grown on trypticase soy agar containing 5% glycerin. After incubation, growth was placed in 2 ml of Maxted's filtrate in a 48° C water bath until the fluid cleared to a point indicating that most of the cells were lysed.

Immune sera were prepared in the following manner. All cultures of Moraxella were grown on Casman's blood agar and the other strains on trypticase soy agar with 5% glycerin. Formalized vaccines, approximating 1,500,000 cells per ml, were made for the inoculation of rabbits in a series of 4 intravenous injections a week for a 2-3 wk interval. Four hours before the first injection of 1 ml, 10 ml of 10% dextrose solution were given intravenously (Holstein, 1948). At the end of 2 wk, all were trial bled and checked. If the titers were 1:1280, the animals were exsanguinated. Most required a 3-wk series of injections.

Eighteen-hour living cultures were used as adsorbing antigens. Two-tenths of a milliliter of cells centrifuged at 2500 rpm for 30 min were added to each ml of indicated antiserum, incubated at 50° C for 1 hr, and centrifuged. The adsorbed sera were tested at a dilution of 1:2 for the precipitin tests and diluted 1:20 through 1:1280 for tube agglutinations. The latter were incubated at 50°C for 18 hr.

For slide agglutinations the antigens were prepared as previously described (Cary et al., 1958). One drop of polyvalent antiserum and one drop of alkali-treated cells resuspended in saline were placed on a spot plate. The plate was shaken for 3 min and read. A live antigen was also prepared for comparison with the alkali-treated cells.

The observation made in the original study (Cary et al., 1958) on the loss of group- and type-specific antigens when the cultures were maintained in the laboratory over long periods applied to the present work. Mice (14-16 gm) were injected intraperitoneally with heavy suspensions of microorganisms grown on blood agar. After 18 hr, they were exsanguinated. From two to twenty mouse passages were required to obtain the results reported here.

RESULTS

In Table 1, the precipitin reactions using Maxted's filtrate are summarized. With pooled Mima polymorpha var. oxidans antiserum, 7 out of 10 strains of Moraxella reacted. In contrast only 2 Moraxellae reacted with polyvalent Mima polymorpha antiserum. The precipitin procedure used here was originally devised in 1956 for the detection of a group antigen in M. polymorpha. At that time only a type-specific antigen was demonstrated with this method. In the current study, the reaction of one Moraxella and a weak reaction of Moraxella non-liquefaciens indicated that these strains share an antigen with one or both of those included in the polyvalent antiserum. From the precipitin tests, a greater serological similarity between the Moraxellae and M. polymorpha var. oxidans was observed. Six strains of Moraxella reacted with Moraxella lacunata antiserum. Bacterium lwoffi var. brevis reacted only with the M. nonliquefaciens and M. lacunata antisera.

Table 1. Precipitin reactions.

Maxted Extracts	Polyvalent Mima polymorpha Antiserum	Mima polymorpha var. oxidans (3 sera)	Moraxella lacunata Antiserum	Moraxella non-liquefaciens Antiserum
Moraxella sp. (5 strains)	1/5 +	5/5 +	3/5+	-
Moraxella lacunata	-	-	+	-
Moraxella bovis	-	+	-	-
Moraxella anapestifer	-	-	+	-
Moraxella nonliquefaciens	±	+	+	+
Bacterium lwoffi var. brevis	-	-	+	±
Mima polymorpha var. oxidans (3 strains)	3/3 +	3/3 +	3/3 +	1/3 +

The slide-agglutination technique with alkali-treated cells which previously has been found as the only means of detecting a group antigen in M. polymorpha (Cary et al., 1958) was employed with Moraxellae for the purpose of demonstrating whether they share a group antigen with the Mimae. Four out of seven Moraxellae were strongly agglutinated by the polyvalent M. polymorpha antiserum and M. polymorpha var. oxidans as indicated in Table 2. When these tests were run, two strains of Moraxella had become too rough to be used. B. lwoffi var. brevis was not agglutinated by either serum. From the results with living suspensions (Table 2), one finds lout of 7 Moraxellae reacted with both antisera.

Using tube agglutinations, the homologous titers were between 1:320 and 1:1280. There were no cross reactions in the Moraxella group or with the M. polymorpha var.

Table 2. Slide agglutinations.

Antigens	Polyvalent *Mima polymorpha* Antiserum	*Mima polymorpha* var. *oxidans* (3 sera pool)
Live Antigens		
Moraxella sp. (3 strains)	-	-
Moraxella lacunata	+	+
Moraxella bovis	-	-
Moraxella anapestifer	-	-
Moraxella nonliquefaciens	-	-
Bacterium lwoffi var. *brevis*	-	-
Mima polymorpha var. *oxidans*	+	+
Alkali-Treated Cells		
Moraxella sp. (3 strains)	+	+
Moraxella lacunata	±	±
Moraxella bovis	-	-
Moraxella anapestifer	±	±
Moraxella nonliquefaciens	+	+
Bacterium lwoffi var. *brevis*	-	-
Mima polymorpha var. *oxidans*	+	+

oxidans antisera. Cross reactions of 1:160 did occur within the M. polymorpha var. oxidans group.

Seeliger (1953) felt that Diplococcus mucosus and Bacterium anitratum were probably the same. Five of his strains were compared serologically to Ferguson's B. anitratum, types 1-10. In the original study (Cary et al., 1958), Ferguson's ten serotypes were agglutinated by polyvalent M. polymorpha antiserum. One of the five strains studied here with alkali-treated cells was strongly agglutinated by polyvalent M. polymorpha antiserum. A strong precipitin reaction was also observed with B. anitratum, type 7. Reciprocal adsorptions were performed with this strain of D. mucosus and B. anitratum, type 7 to ascertain whether they were homologous types. By using tube and slide agglutinations with adsorbed and unadsorbed sera, this strain proved to be the same serologically as B. anitratum, type 7. The remaining D. mucosus cultures were agglutinated by the polyvalent adsorbed Herellea antiserum. These strains also share partial antigens with B. anitratum, types 6 and 9. Alkali-treated cells of N. winogradskyi were agglutinated by polyvalent adsorbed Herellea antiserum, and this culture shares partial antigens with B. anitratum, types 3 and 5.

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REFERENCES

Cary, S.G., R.B. Lindberg and J.E. Faber, Jr. 1956. Typing of Mima polymorpha by a precipitin technique. Jour. Bact. 72: 728-729.

_____, _____, _____. 1958. Slide agglutination technique for the rapid differentiation of Mima polymorpha and Herellea from the Neisserieae. Jour. Bact. 75: 43-45.

De Bord, G.G. 1942. Descriptions of Mimeae tribe with three genera and three species and two new species of Neisseria from conjunctivitis and vaginitis. Iowa State Coll. Jour. Sci. 16: 471-480.

Ferguson, W.W. and L.F. Roberts. 1950. A bacteriological and serological study of organism B5W (Bacterium anitratum). Jour. Bact. 59: 171-183.

Henriksen, S.D. 1952. Moraxella: Classification and taxonomy. Jour. Gen. Microbiol. 6: 318-328.

Holstein, M. 1948. Preparations and reduction in the time of using precipitin sera for detectable ingested blood of insects. Acta Tropica, Basale. 5: 306-326.

Lemoigne, M., H. Girard and J. Jacobelli. 1952. Bacterie du solutilisant facilement le 2-3 butanediol. Annales de L'Institut Pasteur. 82: 389-398.

Seeliger, H. 1953. Zur Systematik des Bacterium anitratum (Shaub and Hauber). Zentralbl. f Bakt. Orig. 159: 173-176.

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CONTRIBUTION TO THE TAXONOMIC STUDIES OF SERRATIA KILIENSIS (LEHMANN ET NEUMANN) BERGEY

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SUMMARY: A study of the morphological, cultural and biochemical features of five strains of Serratia kiliensis (Lehmann et Neumann) Bergey from different culture collections leads to the conclusion that Serratia kiliensis is a variety of Serratia marcescens.

In 1888 a red pigmented microorganism, which was called only bacterium h, was isolated from drinking water by Breunig in Kiel. The name Bacterium kiliense was given to it later by Lehmann and Neumann (1896). This species was placed in the genus Serratia by Bergey (1923) with the name Serratia keilensis (sic). Breed (1957) corrected the spelling of the specific epithet to kiliensis. Some authors (Lehmann and Neumann 1927, Breed 1957, Davis et al., 1957) have suggested that Serratia kiliensis is a variety of or identical with S. marcescens. However, S. kiliensis is still given specific status by authors (Krasilnikov 1949, Breed 1957).

We have therefore undertaken to ascertain whether S. kiliensis and S. marcescens are synonyms.

MATERIAL AND METHODS

We have studied 5 strains supplied as Serratia kiliensis by various culture collections. Strain 274 was from the Department of Agriculture, Ottawa, Canada; strain 300 from Department of Biological Sciences, Purdue University, Indiana; strain 526 from J. Simpson, Prairie Regional Laboratory, Saskatoon, Canada, strains 7462 and 922 from the American Type Culture Collection.

The methods used were the same as in our previous work (Martinec and Kocur 1960).

RESULTS

Morphology. All five strains formed small Gram negative rods, singly or in groups. 0.8 x 1.5-2.0μ. All strains were motile.

Cultural characteristics. Colonies were round with undulate margin, slightly convex, smooth. On nutrient agar two strains only were pigmented. In nutrient broth all strains produced turbidity and sediment, two strains formed a fragile pellicle. On potato, gelatin and on medium Dewey and Poe (1943) only 2 strains were pigmented.

Biochemical characteristics. The ascertained biochemical characteristics are shown in Table 1.

DISCUSSION

No essential differences in morphological and cultural characters were found among the five strains studied. Some minor differences appeared in the biochemical characteristics. Strain 526 hemolysed blood and did not form gas from glucose, strains 582, 274 and 581 fermented lactose. The variability in fermenting lactose and producing gas agrees with the description given by Davis et al. (1957).

In other respects our results agree with the original description (1888) as well as with the data given by Krasilnikov (1949) and Breed (1957). Breed (as well as some other authors) suggests that S. kiliensis is probably identical with Serratia marcescens but does not prove this identity experimentally. He stated that it is uncertain whether the microorganism isolated by Breunig (1888) was a strain of Serratia marcescens not heavily pigmented. Our results confirm this supposition. Breed also states that one of the features differentiating S. kiliensis from S. marcescens is the fact that S. kiliensis does not produce acethylmethylcarbinol. Out of five strains studied only the strain 526 produced acethylmethylcarbinol. Besides this difference, however, we have ascertained no other difference between S. kiliensis and S. marcescens. Comparison of our results with those of Breed (1957), Davis et al. (1957) and with our earlier work (Martinec and Kocur 1959) leads us to the conclusion that S. kiliensis is only a variety of S. marcescens. This conclusion is reached because no production of acethylmethylcarbinol is the only single one of the substantial biochemical

Table 1. Results of biochemical tests with 5 strains of Serratia kiliensis.

Test	Number Positive	Number Negative
Gelatin liquefaction	5	0
Casein hydrolysis	5	0
Nitrate reduction	5	0
Hydrogen sulfide I.	5	0
Hydrogen sulfide II.	0	5
Indole	0	5
Milk	5	0
Hemolysis	1	4
Glucose	5	0
Gas from glucose	4	1
Lactose	3 d	2
Sucrose	5	0
Maltose	4	1
Galactose	5	0
Fructose	5	0
Rhamnose	0	5
Mannose	4	1
Inulin	4	1
Xylose	4	1
Arabinose	0	5
Glycerol	4	1
Adonitol	0	5
Sorbitol	5	0
Mannitol	5	0
Dulcitol	0	5
Starch	0	5
Esculin	5	0
Koser's citrate	5	0
Simmons' citrate	5	0
Acethylmethylcarbinol	1	4
Methyl red	0	5
KCN	5	0
Phenylalanin	0	5
Catalase	5	0
Urease	0	5
Lipase	5	0
5% NaCl	5	0
7.5% NaCl	4	1
Pigment	2	3

Hydrogen sulfide I. = lead acetate papers
Hydrogen sulfide II. = modified Klinger's agar
d = delayed

features which is suitable in taxonomic differentiation of the Serratia genus. According to our opinion the mentioned biochemical test is a more substantial feature than the strain differences in fermenting of carbohydrates or in pigmentation, etc.

From our previous work (Martinec and Kocur, 1961) and from present study it follows that the genus Serratia includes only one species - S. marcescens with the variety Serratia marcescens var. kiliensis.

REFERENCES

Breed, R.S. and M.E. Breed. 1927. The genus Serratia Bizio. Zentralbl. f. Bakt. II. Abt. 71:435-440.

_____, E.G. Murray and R.N. Smith. 1957. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

Breunig, J. 1888. Bacteriologische untersuchung des Trinkwasserss des Stadt Kiel. Diss. Kiel.

Davis, B.R., W.H. Ewing and R.W. Reavis. 1957. The biochemical reactions given by members of Serratia group. Internatl. Bull. Bact. Nomen. and Taxon. 7: 151-160.

Krasilnikov, N.A. 1949. Opredelitěl bakterij i aktinomycetov. Moskva.

Lehmann, K.B. and R.O Neumann 1886. Bakteriologische diagnostik. München.

Martinec, T. and M. Kocur. 1960. The taxonomic status of S. plymuthica (Lehmann and Neumann) Bergey et al. and of S. indica (Eisenberg) Bergey et al. Internatl. Bull. Bact. Nomen. and Taxon. 10(4): 247-254.

_____, _____. 1961. The taxonomic status of Serratia marcescens Bizio. Internatl. Bull. Bact. Nomen. and Taxon. 11(1): 7-12.

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RESULTS OF A COMPARATIVE STUDY OF CRITERIA USED IN THE CLASSIFICATION OF THE ACTINOMYCETES

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SUMMARY: The results of a comparison of criteria used in characterization of the Actinomycetes under the auspices of the International Subcommittee are reported. Although this first co-operative work carried out on a large scale showed some deficiencies which were caused by the selection of the media and methods and by the type of description, some interesting evidence concerning the value of the used criteria for the classification of Streptomycetes were obtained.

The morphology of sporophores and the shape of spores are significant and constant characteristics so that they can be used as taxonomically useful criteria.

Likewise, the melanin reaction is an unequivocal characteristic and can be applied in a classification. The determination and description of the colours of the aerial as well as of the vegetative mycelium are much more complicated. In principle these results confirm those obtained from a co-operative experiment which was carried out in the USA a short time before (Gottlieb, 1951). In this study organized by the Subcommittee on Actinomycetes of the Committee on Taxonomy of the Society of American Bacteriologists workers ot 11 different laboratories examined 5 different strains by means of 12 morphological as well as physiological criteria

under strictly defined methods.

Further studies are necessary to find the most useful criteria and to improve the methods which are suitable for the classification of Streptomycetes. Further co-operative and comparative work could be the best way to solve these taxonomical problems.

During the course of the Round Table Conference on Streptomycetes in Stockholm in August 1958, the participants formulated a plan for a cooperative experiment which should establish the reproducibility of the stable properties used in the characterization of Streptomycetes. In accordance with this plan (Küster, 1959) an international co-operative work has been carried out. Thirty two individuals participated in this experiment. In twenty nine laboratories 25 strains from Prof. Baldacci's collection were examined, and three other laboratories used 10 strains only. The experiments were carried out three times at definite time intervals; only one laboratory attempted them once.

The main tasks of the co-operative study were first of all to decide whether a characteristic was sufficiently constant and reproducible to be used for classification and secondly to standardize the type of description as much as possible so that the individual observations could become comparable. Both questions have not been solved completely but the results may be sufficiently interesting to justify publication.

The following criteria were employed and examined:

1) Morphology of sporophores: The different forms were described according to the nomenclature of T.G. Pridham et al. (1958) with only a few modifications as proposed by Baldacci. The morphology of sporophores is a significant and constant characteristic. Nineteen of the 25 test strains were determined unequivocally and in the same way. The differentiation between spiral and non-spiral was quite easy. Those between Flexibilis and Retinaculum and between Rectus and Flexibilis respectively seemed to be not so simple. Possibly the scheme used for the determination of the morphology of sporophores as one of the main characteristics with taxonomical value could be simplified for future studies which would lead to a division into groups, series, and the like.

2) Form of spores: The examination of the spores by means of an electron microscope was carried out in 22 lab-

oratories. The results were clear and definite. The differentiation between smooth and spinous spore surfaces was simple. Dissenting observations were very rare. This shows that the shape of spores is also a significant and constant characteristic so that it can be used as a taxonomically useful criterion.

3) Melanin-reaction: A 100% or nearly 100%-decision for positive or negative was very frequent. An uncertainty occurred very seldom. In the most extreme case the deviation was not more than 16%. The negative melanin reaction was distinctly easier to determine than a positive reaction which always also showed deviations. Consequently the melanin reactions seems to be an unequivocal characteristic which can be applied in a classification becasue of its advantage of showing only two alternatives of the determination (+ or -). Intermediates of doubtful determination are rare. In the opinion of some workers (Tresner and Dange, 1958, Hütter, 1961) these intermediates can be eliminated by replacing the melanin reaction by the H_2S reaction. This reaction gives results which are almost always in accord with those of the melanin reaction and can be described more clearly and more unequivocally as positive or negative.

4) Colour of the aerial mycelium: A clear determination of the colour of the aerial mycelium is very difficult so that one dominant colour could be determined with only 17 strains. The colour of the aerial mycelium is very rarely so clear that it can be classified into one of the 9 suggested colours, white, yellow, orange, pink, red, blue, green, gray, brown. Mostly various intermediates and shades occurred which were allocated to two or more basic colours. Furthermore, the determination of colour is rather a personal matter, more or less subjective. Each observer has not only a different personal perception of colours, but he may also determine the same culture differently at different times, particularly when dealing with cultures showing coloured intermediates which are termed white one day and gray another. The kind of light is also important, bright or gloomy daylight or artificial light influencing the determination of colour. The difficulty in determining the colour is more a matter of describing and interpreting than of the variability of the colour. In my opinion the variability of this characteristic is not the main reason for this complicated situation. The subjective determination is too variable, and an exact stan-

dardizated description was not possible under the conditions of this experiment. The use of standard colour plates with code numbers may partly eliminate these difficulties because they also comprehend the intermediates, but the subjective manner of observing and describing is not completely excluded. For the division into larger taxonomical units such as groups, series, and the like it is necessary to fix which colour numbers, i. e. which intermediates, should be attached to one basic colour. The best solution might be a method by which the colour would be determined objectively, e. g. by spectrophotometric measurements. N. Paulin (1960) started some experiments using this technique. Although the results are not yet sufficiently satisfying they may be the basis for further studies in this direction.

5) Colour of the vegetative mycelium: All the above mentioned facts which make a determination of the colour of the aerial mycelium so difficult occur to a much greater extent in determining the colour of the vegetative mycelium. Here, a dominant colour could be chosen with only 9 strains. In some cases up to 9-10 different colours were observed. It may be interesting to note that some of these strains also showed the greatest variation in the colour of the aerial mycelium. Even more than for the determination of the morphology which is constantly and equally distinct on all substrates, the selection of suitable media is important for the determination of the colour. Because of different requirements of the individual species and strains it is difficult to find one optimal medium which produces a strong aerial mycelium with distinct colour in all cases and makes an observation of the colour of the reverse side of the vegetative mycelium also possible. But for the determination of a taxonomic characteristic it is absolutely necessary to apply the same medium always even if it is not optimal for all strains. This does not exclude the additional description of the growth on a special medium, in particular if it is quite different from that on the standard medium.

One of the most important tasks of the co-operative work was to decide whether a characteristic was sufficiently constant to be used for classification. In our case all the observations were carried out three times at definite time intervals of two months. It might be interesting to state which observation produced the greatest number of dissenting results. An observation different from the dominant deter-

mination of one strain by one worker is termed dissent. In this way the first observation can also be a deviation. The other alternative of adopting the first observation always as the basic one has not been taken into consideration. Table 1 indicates the sum of deviations in all strains with regard to each characteristic.

Table 1. Aberrations of the dominant observation.

Characteristic	Observation 1st	2nd	3rd	Totals
Morphology of sporophores	48	38	59	145
Forms of spores	--	4	1	5
Colour of aerial mycelium	80	49	60	189
Colour of the vegetative myclium	96	68	64	228
Totals	224	159	184	567

This confirms the above explained variations in the observation of the characteristics, inter alia, that the determination of the colour of the vegetative mycelium shows the greatest degree of variation. The greatest number of deviations with regard to colour determination is recorded in the first observation, but this need not be caused by a variation of the colours within the subsequent observations. It might also be suggested that the observer got accustomed to the proposed scheme during the repeated observations.

It may always be difficult to divide the natural appearances which occur as various intermediates into a hard and fast scheme. Only by many individual observations can a large sample of figures be obtained which can be evaluated statistically so that the dominant characteristic can be seen more or less distinctly. In this way a 100%-decision was given if one worker unequivocally observed only one form or colour respectively within one strain. In the other cases the critical examination was not 100%, but only 50, 33, 25, 20% etc. The following statement may also be interesting to demonstrate whether the so-called 100%-decision originated always

from the same worker. Most of the workers could classify their observations concerning the morphology of sporophores into the scheme more easily than their determination of the colours. Some workers gave a 100%-decision very frequently with each characteristic, but this must not mean that they are less critical than the others but only that they noted the dominant tendency as determination on the filing cards.

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REFERENCES

Gottlieb, Dr. 1961. An evaluation of criteria and procedures used in the description and characterization of the Streptomycetes. Appl. Microbiol. 9: 55-65.

Hütter, R. 1961. Zur Systematik der Aktinomyceten. V. Die Art Strept. albus (Rossi-Doria emend. Krainsky) Waksman et Henrici 1943. Arch. Microbiol. 38: 367-383.

Küster, E. 1959. Outline of a comparative study of criteria used in characterization of the Actinomycetes. Internatl. Bull. Bact. Nomen. and Taxon. 9: 97-104.

Paulin, N. 1960. La valutazione objettiva del colore nelle colonie di Attinomiceti. Giorn. Microbiol. 8: 91-114.

Pridham, T.G., C.W. Hesseltine and R.G. Benedict. 1958. A guide for the classification of Streptomycetes according to selected groups. Appl. Microbiol. 6: 52-79.

Tresner, H.D. and F. Danga. 1958. Hydrogen sulfide production by Streptomycetes as a criterion for species determination. Jour. Bact. 76: 239-244.

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THE DEFINITION OF PHAGE TYPES OF SALMONELLA PARATYPHI B

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SUMMARY: A phage type is defined as a collection of bacterial strains with the same phage relationships. Satisfactory type definitions have been developed for Salmonella typhi. Definition for type definitions for S. paratyphi B are discussed.

Phage typing results in a varietal subdivision of Salmonella (species, Kauffmann 1959) into phage types.

A phage type is a collection of bacterial strains with the same phage relationships.

Before taxonomy of types can be discussed a definition of type is necessary. The application of this type definition should give rise to a systematic arrangement of types, if possible. The conditions which a collection of bacterial strains must satisfy if such collection is to be recognized as a phage type must be a guarantee of the homogeniety of the collection. These conditions must be satisfied objectively and must be reproducable.

Such a type definition results for Salmonella typhi. A new type is being determined by and adaptation of a Vi phage, and, as far as possible by an adaptation of a phage Vi II of Craigie and Yeh (1938). The homogeneity of the phage types was confirmed by an examination of the lysogenic properties.

The relationship is different with S. paratyphi B. A similar type definition is not given in the system of Felix and Callow (1951). The role of the adaptations of phage 1 of Felix and Callow is different from that of phage Vi II in the system of Craigie and Yen. It has appeared that the adaptations of phage 1 of Felix and Callow indicated groups of types (Scholtens, 1955). These groups can be subdivided with specific phage reactions into types as is done in the natural system of phage types of Salmonella paratyphi B (Scholtens, 1956, 1959).

With the natural system of "phage types" a type defini-

tion results for the phage types of Salmonella paratyphi B; all bacterial strains of a natural phage type possess the same phage sensitivities besides the same lysogenic properties (Scholtens, 1952). The homogeneity of one of the group of properties is a guarantee of homogeneity as regards the other. This type definition results in a systematic arrangement of types (Scholtens, 1956). It covers all the phage relationships (properties) of the bacterial strains. It agrees with the International Code of Nomenclature of the Bacteria and Viruses that recognized the individual micro-örganism as a unit.

LITERATURE

Craigie, J.A. and C.H. Yen. 1938. Can. Publ. Hlth. Jour. 29: 448.

Felix, A. and B.R. Callow. 1951. The Lancet, II, 3.

Kauffmann, F. 1959. Internatl. Bull. Bact. Nomen. and Taxon. 9:1

Scholtens, R. Th. 1952. Ant. v. Leeuwenhoek. 18: 257.

_____. 1956. Ant. v. Leeuwenhoek. 22: 65.

_____. 1959. Ant. v. Leeuwenhoek. 25: 403.

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A SYSTEM OF PATHOGENIC LEPTOSPIRAE ISOLATED IN THE U.S.S.R.

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SUMMARY: A proposal of a system of classification of the pathogenic species of the bacterial genus Leptospira based on the standard strains found in the culture collections of Prof. Wolff in Amsterdam and of the Metschnikoff Institute of Vaccines and Sera in Moscow. For the species L. icterohaemorrhagiae one serogroup and four serotypes are listed with names and for L. febris aquatilis five serogroups and twelve serotypes.

The classification of the pathogenic leptospirae is not yet accomplished; the solution of this problem is now in the stage of the systematization of the leptospirae.

In recent years work in this direction has been conducted in a number of countries (Wolff, Broom, Varfolomeeva, Terskih, Baburieri et al.).

Comparison of pathogenic leptospirae isolated in the Soviet Union with foreign standards has been carried out by means of serological methods (agglutination-lysis test, agglutinin-absorption test) and also by use of biological methods.

As a result of these investigations we are proposing a system of leptopsiral characteristics and origins for the organisms that are sufficiently known (Table 1).

The sequence listed below consists of four taxonomic categories and designations of standard strains of leptospirae found in the culture collection of Prof. Wolff (Amsterdam) and in that of the Metchnikoff Institute of Vaccines and Sera (Moscow).

1. Genus Leptospira Noguchi, 1917. The fact that pathogenic as well as saprophytic leptospirae are to be included in the genus Leptospira is not questioned.

2. Species of Leptospira. Species of Leptospira exist as an objective reality. However, marked distinctions both in specific and in fermentative properties are as yet not sufficiently investigated, all of which complicates the determination of species in these microorganisms.

Table 1.

Genus	Species	Serogroup	Serotype	Strains
Leptospira (Noguchi, 1917)	L. icterohaemorrhagiae (Inada, Ido, Hoki, Kaneko, Ito, 1916)	icterohaemorrhagiae	L. icterohaemorrhagiae (AB)	Wijnberg Leningradsky-Sudjin Belousov
			L. icterohaemorrhagiae (A)	Kantorowicz Odessa 8-57 Jablochkin
		--------	L. canicola	Utrecht IV Makarov
		--------	L. sorex* (Varfolomeeva and Nikiforova, 1947)	Javanica Poi Sorex
	L. febris aquatilis (nom. nov. Tokarewitch, 1954)	grippotyphosa	L. grippotyphosa (Tarassoff, 1928)	Dmitrovsky Kornilov Vitulina
		pomona	L. pomona	Pomona Manjakow a sucking pig -2
		tarassovi	L. tarassovi (Tarassoff, 1938)	T-384 Mitis Johnson
		bataviae	L. bataviae	van Tienen Micromys minutus 167
		hebdomadis	L. hebdomadis	Akiyami B Terehov
			L. saxkoebing	Mus 24 P-183 Nero

	L. ussuri (Kraminskaya, 1959)	DW-P
	L. muris (Kraminskaya, 1956)	BM-1
Kazachstanika I	L. kazachstanika I (Krepkogorskaya, 1955)	№ 154 № 184
?	L. kazachstanika II** (Krepkogorskaya, 1955)	Kaz II
?	L. erinacei auriti** (Ananjin, 1951)	Erinacei auriti
?	L. erinacei europaei** (Ananjin, 1953)	Erinacei № 1 (23)

* The name of the author and the year of isolation are given only for those types of leptospirae which were first established in the Soviet Union.

** These types being quite independent are in the process of further investigations.

In the U.S.S.R., on the basis of the study of the antigenic structure of leptospirae, clinical picture, epidemiology of leptospirosis and also data on their evolution, two concepts as to species in leptospirae have been developed which have come into practical use in scientific and other laboratories (Varfolomeeva, 1947). Improvements in the methods and techniques used for this purpose may involve further changes.

Determination of taxonomic categories in the leptospirae is based on the stability of their antigenic structure. Each species is divided into serogroups and serotypes.

3. Serogroup. A serogroup includes two or more serological types possessing one related antigen and also serotypes having other antigens in addition to this common antigen.

We regard a serogroup not as a replacement of the genus Leptospira but as a certain transitional stage for further delimitation of species in this genus. A species can embrace one or more serological groups.

While dividing leptospirae into serogroups one should attempt to expand them. In our opinion serogroup icterohaemoorhagiae can be enlarged by adding as serotypes L. canicola and L. sorex (see broken lines in the table) or by others closely related as to their antigenic structure.

4. Serological type. In the column of the table under this heading all serological types known so far in the U.S.S.R. are listed. Fourteen of them isolated from human beings have already been investigated. The serotypes are given with their conventional specific binominal names. In the 5th column names of standard foreign and local strains of the leptospirae are indicated.

REFERENCES

Babudieri, B. 1960. Die Zellstruktur und die Serologie der Leptospiren. Ergeb. Mikrobiol. Immunitätsforsch. exp. Ther. 33: 260-306.

Breed, R., E.G.D. Murray and N.R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

Kauffmann, F. 1959. On the principles of classification and nomenclature of Enterobacteriaceae. Internatl. Bull. Bact. Nomen. and Taxon. 9: 1-7.

Terskich, V.I., T.F. Sakhartseva and Z.H. Karimova. 1960. The comparative serological study of some type strains of leptospiraeisolated in the Soviet Union and abroad. Theses of reports of the 3rd conference "Problems of leptospirosis". p. 10.

Varfolomeeva, A.A. 1949. Leptospiral infections in men. Microbiology.

_____. 1958. The origin, properties and nomenclature of L. monjakow, a causative agent of leptospirosis. Jour. Microbiol. Epidem. Immunol. 8: 36-42.

_____. 1958. The properties and nomenclature of L. perepelizin, a causative agent of benign Leptospirosis. Jour. Hyg., Epidem., Microbiol. and Immunol. II: 50.

_____. 1958. Charakteristik und Nomenklatur des Erregers des Leptospirosen. L. monjakow. Zentralbl. Bakt. I. Orig. 171: 71-75.

Wolff, J.W. 1954. The laboratory diagnosis of leptospirosis. Springeld, Ch.C. Thomas.

_____ and J.C. Broom. 1954. Problems of classification and a suggested System based on antigenic analysis. Docu. Med. geograph. trop. Vol. 6.

Varfolomeeva, A.A. 1957. Jour. Microbiol. Epidem. Immunol. 1:.

_____ and A.S. Nikiforowa. 1949. Jour. Microbiol. Epidem. Immunol. 3:.

Tarassoff, S.I. and H.W. Epstein. 1928. Hygiene and Epidemiology. 9: 51-52. (Russ.)

Kraminskaya, N.N. 1959. Jour. Microbiol. Epidem. Immunol. 3:.

_____ and V.A. Eskin. 1956. Jour. Microbiol. Epidem. Immunol. 9:.

Krepkogorskaya, T.A. 1955. The natural hearth of diseases of men and regional epidemiology. L.

Ananjin, W.W. 1951. Jour. of Zool. t. XXX 4: 375-376.

_____. 1953. Jour. Microbiol. Epidem. Immunol. 4:.

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BACTERIUM ANITRATUM TRANSFERRED TO THE GENUS CYTOPHAGA*

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SUMMARY: Bacterium anitratum Schaub and Hauber is transferred to the genus Cytophaga (Myxobacteriales, Schizomycetes) as Cytophaga anitrata (Schaub and Hauber) comb. nov. The transfer is based upon cellular morphology, the characteristic gliding or creeping motility of the single cells and the absence of microcysts. The nearly related Moraxella lwoffi Audureau is likewise transferred to Cytophaga as Cytophaga lwoffi (Audureau) comb. nov. for the same reasons.

In recent years several papers have been published on Bacterium anitratum Schaub and Hauber 1948 and different proposal made for its classification. It has been placed in at least five different bacterial families but none of the taxonomic proposals made so far have been entirely satisfactory A new proposal is advanced here based on the observation that this microorganism exhibits the special kind of motility found in myxobacteria and described as gliding or creeping.

Gliding motility has been demonstrated in 10 out of 15 strains studied in detail, including an authentic B5W strain from Stuart. The observations were performed on cultures growing on thin agar plates poor in nutrients by aid of a phase-contrast microscope.

The motion is slow, hesitating and intermittent as in other myxobacteria and the phenomenon is an erratic one. As yet it has not been possible to find conditions which will regularly allow of its demonstration. This is inconvenient in diagnostic work but does not alter the significance of the phenomenon in taxonomic considerations.

Higher myxobacteria form resting cells called microcysts

*Summary of a paper read at the 13th Scandinavian Congress for pathology and microbiology at Åbo, Finland, June 1961.

which are sometimes carried in specialized fruiting bodies. In Bacterium anitratum such structures have not been observed and therefore—according to Stanier's classification of the order Myxobacterales in Bergey's Manual, 7th Ed. — the organism should be assigned to the genus Cytophaga, which accommodates all single celled bacteria which exhibit gliding motility but do not form fruiting bodies or microcysts.

It is consequently proposed that Bacterium anitratum be transferred to the order Myxobacterales, family Cytophagaceae, and placed in the genus Cytophaga and designated as Cytophaga anitrata (Schaub and Hauber) comb. nov.

Besides Bacterium anitratum a few strains of the very imilar organism, originally described as Moraxella lwoffi Audureau 1940, have also been examined and found to possess the same ability for gliding motility as Bacterium anitratum. At present it is not clear whether both of these closely related organisms deserve specific rank, but if it is eventually recommended to establish two species, Moraxella lwoffi Audureau would become Cytophaga lwoffi comb. nov.

REFERENCES

Audureau. 1940. Ann. Inst. Past. 64: 150.

Schaub, I. G. and F. D. Hauber. 1948. A biochemical and serological study of a group of identical unidentifiable Gram-negative bacilli from human sources. Jour. Bact. 56: 379-385.

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EDITORIAL NOTE

The following Letter to the Editor was received too late for insertion in the April issue of the INTERNATIONAL BULLETIN. Although the date for receiving applications for allocations through the International Association of Microbiological Societies was fixed at May 15, 1961, publication is this (July) issue of the INTERNATIONAL BULLETIN is believed advisable to give publicity to the method of subvention allocation that has been adopted, and to emphasize from the standpoint of systematic bacteriology the importance of adequate support for important culture collections.

The Editorial Board of the INTERNATIONAL BULLETIN wishes to emphasize the importance of the request from the Secretary of the International Association of Microbiological Societies published in this issue of the BULLETIN. One of the important factors in the simplification and unification of bacteriological nomenclature is the designation of true type cultures of species, subspecies, and infrasubspecific forms. If there are true type cultures in existence they should be recognized. If there are cultures that bear the names of species that cannot qualify as type cultures under the International Code of Nomenclature of the Bacteria and Viruses, they should be recognized as possible candidates for designation as neotype cultures. A type or neotype culture is one which is recognized as a standard and is available to all workers for comparison. The need for such standard or type cultures is increasingly apparent; they are quite as necessary in bacteriology as in zoology and botany where types specimens are universally recognized as basic to stability in taxonomy and nomenclature.

LETTER TO THE EDITOR

To the Editor of the International Bulletin of Bacteriological Nomenclature and Taxonomy:

On behalf of the Executive Committee of the International Association of Microbiological Societies I request your cooperation in making known to your readers the possibility of financial support for the international activities of persons and institutes maintaining culture collections. Considering

the fact that the deadline for this year's applications has to be set at May 15th I would appreciate if you insert the following notice in your journal as soon as possible.

"Collections of Micro-organisms exist in many countires of the world, some are financed by a government department, others by universities or research institutes. Many work on small budgets and could improve the services they render if they had a bigger income. Some collections make a charge for cultures, others issue them free of charge.

Culture Collections, particularly specialised Collections containing strains not available elsewhere, often serve international ends by making these strains and specialised knowledge available to workers in other countries, and in doing so incur expences they have difficulty in meeting. The executive Committee of the International Association of Microbiological Societies (IAMS) acts as an advisory body in the allocation of whatever funds are available for furthering the international activities of such Collections. The amount of money is at present limited, but in order that the Committe's advice may be as informed as possible, it is essential for IAMS to have details of Collections at present distributing cultures in this way, including their financial dependence on external grants.

It is suggested, therefore, that curators or directors of culture collections should notify the secretary of the Executive Committee of IAMS (Dr. C. -G. Hedén, Karolinska Institutet, Stockholm 60) of their collection, stating its purpose, the type(s) and numbers of organisms kept, special interests (e.g. medical bacteriology, industrial fermentations.etc.), whether cultures are charged for, the general financial state of the collection, (whether subsidised by the state, industry, etc.) and finally the amount of international interest in their collections, and to what degree they are unable to meet international demands as fully as they would like because of the expenditure involved. The statement must reach IAMS' Stockholm office before May 15th in order to be considered this year."

Sincerely yours,

C.-G. Hedén